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P01/7700 0,00-0318704.4

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1. Your reference

P.89550 TJD

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3. Full name, address and postcode of the or of each applicant (*underline all surnames*)
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Patents ADP number (*if you know it*)

If the applicant is a corporate body, give the country/state of its incorporation

UNITED KINGDOM

8592917001

4. Title of the invention

CHIMAERIC VECTOR SYSTEM

5. Name of your agent (*if you have one*)
 "Address for service" in the United Kingdom  
 to which all correspondence should be sent  
*(including the postcode)*

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Country

Priority application number  
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Number of earlier application

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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

Yes

- a) any applicant named in part 3 is not an inventor, or
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# Patents Form 1/77

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Description	26
Claim(s)	2
Abstract	1
Drawing(s)	2 <i>X 2 LM.</i>

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### Priority documents

### Translations of priority documents

### Statement of inventorship and right to grant of a patent (Patents Form 7/77)

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### Request for substantive examination (Patents Form 10/77)

### Any other documents (please specify)

11.

I/We request the grant of a patent on the basis of this application.

Signature *J.A. Kemp & Co.* Date 8 August 2003  
J.A. KEMP & CO.

12. Name and daytime telephone number of person to contact in the United Kingdom

Timothy J. Duckworth  
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## CHIMAERIC VECTOR SYSTEM

### Field of the Invention

This invention relates to vectors and their use in gene transfer. The vectors  
5 are based on retroviruses, adapted so that they cannot package their own RNA, and  
which can be used as infectious agents to transfer foreign genes, e.g. for somatic  
gene therapy.

### Background of the Invention

10 Use of modified viruses to deliver genetic material to cells, both for research/development purposes and for clinical purposes, is known. Some of the most successful gene transfer systems ('vectors') are based on retroviruses, and more recently, on lentiviruses, a subfamily of retroviridae. Retroviral vectors have the advantages of being able to efficiently infect a broad range of cell types, and of being  
15 able to integrate the genetic material they carry (eg exogenous therapeutic genes) into the genome of the target cell (eg cells of the human patient). However, retroviral vectors can only infect dividing cells, and this limits their use.

Lentiviral vectors have a number of advantages over retroviral vectors including the ability to infect both dividing and non-dividing cells. However, for  
20 both retroviral and lentiviral vectors there are concerns that the genetic homology between the packaging constructs and the constructs comprising the packageable vectors and/or other viral sequences, including sequences present in the cells in which the retroviral vectors are produced, could lead to recombination events that could generate a dangerous replicating virus.

25 These recombination events are particularly prone to occur in the cell line in which the vector is produced. This is because, in order for the cell line to produce the vector, it must contain certain viral sequences which express the proteins and other factors necessary to package the vector into a virus-like particle that then can infect cells, reverse transcribe RNA and integrate the proviral DNA into the host cell  
30 genome. Recombination between the vector and these 'helper' sequences may in theory produce a dangerous replicating virus.

### Summary of the Invention

According to the present invention, a provirus is capable of producing SIV proteins but is not replication-competent because the RNA cannot be packaged into virions. Using this packaging-defective provirus vector, packaging-defective cell lines can be produced that can be used as a system for efficiently introducing a desired gene or genetic sequence into mammalian cells using HIV-2 vectors comprising a HIV-2 packaging signal and heterologous gene sequence.

Accordingly the invention provides a process for producing a Simian Immunodeficiency Virus (SIV) encoding a heterologous gene, which process comprises infecting a host cell with a first vector which is capable of producing SIV capsid and a second vector comprising a Human Immunodeficiency Virus type 2 (HIV-2) packaging signal sufficient to package the vector in the SIV capsid and a heterologous gene capable of being expressed by the vector; and culturing the host cell.

The invention also extends to a virus produced by the processes of the invention. Pharmaceutical compositions may be formulated which comprise such viruses.

The viruses of the invention may be used in gene therapy. Thus, the invention provides a method of delivering a therapeutic or antigenic protein or peptide to an individual comprising administering to the individual an effective amount of a first and second vector as described above, a virus according to the invention or a pharmaceutical composition according to the invention.

### Description of the Figures

Figure 1 illustrates cross packaging data of Gag-pol from HIV-1, HIV-2 and SIV and gene transfer vectors based on HTV-1, HIV-2 and SIV expressing GFP, providing a semi-quantitative PCR analysis of viroin packaged Green Fluorescent Protein (GFP) RNA.

Figure 1A. Semi quantitative PCR analysis of Virion packaged GFP RNA  
 - Marker Kb+ (Invitrogen)  
 1-4 HIV-1 gag-pol + HIV-1 Vector      Limit of PCR +ve dilution      10<sup>3</sup>  
 5-8 HIV-1 gag-pol +SIV vector      Limit of PCR +ve dilution      10<sup>2</sup>

9-12 HIV-1 gag-pol + HIV-2 Vector	Limit of PCR +ve dilution	$10^3$
13-16 SIV gag-pol + SIV Vector	Limit of PCR +ve dilution	$10^2$

- Lanes 17 and 18 are negative controls and 19 is +ve GFP control

Figure 1B. Semi quantitative PCR analysis of Virion packaged GFP RNA

5 Marker Kb+ (Invitrogen)

1-4 SIV gag-pol + HIV-2 Vector	Limit of PCR +ve dilution	$10^4$
5-8 HIV-2 gag-pol +HIV-2 vector	Limit of PCR +ve dilution	$10^3$
9-12 HIV-2 gag-pol + HIV-1Vector	Limit of PCR +ve dilution	$10^2$
13-16 HIV-2 gag-pol + SIV Vector	Limit of PCR +ve dilution	$10^2$

10 Lanes 17 and 18 are negative controls and 19 is +ve GFP control

**Figure 2** FACS analysis of 293T cells transduced with cross-packaged Vectors  
 Illustrates the relative transduction efficiencies of cross packaging vectors in 293T cells by FACS analysis.

15 Key:

HIV-1 gp + HIV-1 GFP:

HIV-1 Gag-pol + HIV-1 GFP gene transfer vector

HIV-1 gp + HIV-1 cppt GFP:

HIV-1 Gag-pol + HIV-1 GFP gene transfer vector containing cppt fragment

20 HIV + DB:

HIV-1 Gag-pol + HIV-2 GFP gene transfer vector

HIV+ SIV:

HIV-1 Gag-pol + SIV GFP gene transfer vector

SIV + cVig:

25 SIV Gag-pol + SIV GFP gene transfer vector

SIV + GFP:

SIV Gag-pol + HIV-1 GFP gene transfer vector

SIV + DB:

SIV Gag-pol + HIV-2 GFP gene transfer vector

30 HIV-2 +DB:

HIV-2 Gag-pol + HIV-2 GFP gene transfer vector

HIV-2 + GFP:

HIV-2 Gag-pol + HIV-1 GFP gene transfer vector

HIV-2 + cVg:

HIV-2 Gag-pol + SIV GFP gene transfer vector

Series 1 to 4 represent data from range of viral vector concentration (20ng, 10ng,  
5 5ng, 2ng, respectively)

### Description of the Invention

Packaging-defective proviral constructs, that is systems in which the provirus  
is capable of producing some viral proteins but is not replication-competent because  
10 the viral RNA cannot be packaged into virions, may be used to create packaging cell  
lines. The packaging defective proviral construct or constructs are known as the  
'packaging constructs. They do not contain on their RNA transcripts the sequences  
required for recognition and encapsidation into a viral particle. Introduction or  
expression of RNA transcripts containing the necessary packaging signal sequences  
15 into packaging cell lines results in that RNA being packaged into virions.

Lentiviral vectors for gene therapy are produced by packaging a gene transfer  
vector with a Gag precursor protein and the incorporation of a heterologous  
envelope. The level of sequence similarity between third generation gene transfer  
vectors and their packaging constructs is kept to a minimum to avoid recombination  
20 events and the possible generation of a replication competent virus. Some primate  
lentiviruses can cross-package each others genomic RNA and a lentiviral vector  
based on two separate viruses may have increased safety. Crucially, the cross-  
packaging relationship is not always reciprocal between viruses and the efficiency of  
gene transfer needs to be similar to the homologous vector system. Previous  
25 published work identified non reciprocity between HIV-1 and HIV-2 however this  
was ascribed to differences in RNA capture mechanisms between the two viruses and  
the difference in the site of their packaging signals relative to the major splice donor.  
The surprising finding in this application is that a non reciprocity exists between  
HIV-2 and SIV such that SIV Gag proteins can capture HIV-2 RNA vectors but that  
30 the reverse cannot occur. This observation is unexplained and could not have been  
predicted from the previously published data or knowledge on the packaging systems  
in the two viruses.

The present invention is based on studies looking at the cross packaging relationship between gene transfer and gag-pol constructs based on human immunodeficiency viruses type 1 and type 2 (HIV-1 and HIV-2) and Simian immunodeficiency virus (SIV).

5 We have shown that:-

- (i) HIV-2 helper sequences do not package SIV vectors
- (ii) SIV helper sequences package HIV-2 vectors.

In particular, we have shown that helper sequences derived from SIV enable the packaging of HIV-2 RNA at high levels and permit efficient gene transfer by the 10 packaged HIV-2 vectors. This last point is important since the utility of a viral vector is dependent on the efficiency with which it can infect target cells. None of these results were predictable from previously published observations.

The utility of the above-mentioned inventive step lies in combining:

- (i) the safety of a vector system in which helper and vector sequences are derived from two different viruses (resulting in very low probability of recombination); and
- (ii) the general advantages of lentiviral vectors; and
- (iii) the specific advantages of HIV-2 and SIV sequences

in a vector system that has a transduction efficiency comparable to the best of other 20 lentiviral systems.

Furthermore, as no HIV-1 or 2 structural proteins are expressed in constructs according to the present invention, there is no risk of AIDS.

In one embodiment of the present invention, one can envisage a producer line containing the gag-pol sequences from SIV and a vector (ie a sequence of nucleic acid containing a packaging signal) derived from HIV-2. The producer line would 25 also contain a sequence encoding an envelope protein which could come from a variety of sources including, but not limited to, ecotropic retroviruses, amphotropic retroviruses, vesicular stomatitis virus (VSV), any other kind of virus, and any other proteins including but not limited to antibodies and antibody-like molecules, and any

epitopes or sub-units thereof, and any modifications or fragments of any of the above including but not limited to general post-translational protein modifications such as glycosylation (which might be dependent on the expression of native or exogenous glycosyltransferases or glycosylases or other enzymes in the producer line or in the supernatant).

The affinity for target cells of this gene transfer system would be provided by the envelope sequences, while the efficient reverse transcription and integration functions would be provided by the SIV gag-pol. The efficient packaging would be provided by a combination of the SIV gag-pol and the HIV-2 vector sequences. This combination would give rise to superior gene transfer efficiency as compared with many other systems. In addition, the derivation of gag-pol and vector sequences from SIV and HIV-2 (and an envelope gene usually from a third source) respectively would make the recombination probability very low, ensuring that the system is safer than other viral vector systems. The superior safety and efficiency features derived from the combination of SIV and HIV-2 sequences would be combined with the general advantages of lentiviral vectors (eg stable, long-term expression in dividing and non-dividing human cells and minimal disruption of the endogenous human genetic material) to give a gene transfer system of superior safety, efficiency and stability of expression in human cells.

20

#### Packaging defective SIV vectors

It is possible to make SIV packaging-defective vectors. The region between the primer-binding site and the 5' major splice donor in SIV contains sequences necessary for efficient packaging of SIV RNA into virions (Strappe, P.M., Greatorex, J.S., Thomas, J, Biswas, P, Lever A.M.L. *The major packaging signal of SIV is upstream of the splice donor at a similar distance from the cap site as those of HIV-1 and HIV-2* *J.Gen Virol* 2003 84:2423-2430). In addition, the region between the 5' major splice donor and the *gag* initiation codon contains a second and less important region, important but not essential for packaging of SIV RNA into virions. One can prepare a vector comprising a packaging-defective SIV provirus, wherein the vector contains a nucleotide sequence which corresponds to a sufficient number of nucleotides from an SIV genome to express desired SIV products, but does not

correspond to a sufficient number of nucleotides corresponding to the region between the primer-binding site and the 5' major splice donor or between the splice donor and the *gag* initiation codon to efficiently package SIV RNA (the packaging sequence).

These sequences preferably correspond to the genome of SIV. The term  
5 corresponds means that conservative additions, deletions and substitutions are permitted. The primer-binding site (23 bp) and the 5' major splice donor are respectively numbered 121-143 and 295-296 in the genomic nucleotide sequence where the transcript start site is defined as 1.

In particular, an SIV genome as used herein refers to the viral RNA derived  
10 from an SIV. The simian immunodeficiency viruses (SIV) of the invention may be derived from any SIV strain, or derivatives thereof. Derivatives preferably have at least 70% sequence homology to the SIV genome, more preferably at least 80%, even more preferably at least 90 or 95%. Other derivatives which may be used to obtain the viruses of the present invention include strains that already have mutations  
15 in some SIV genes such as a mutation in the *nef* gene as described in Rud et al 1994 J. Gen Virol 75, 529-543. Other mutations may also be present as set out in more detail below. The position of the primer binding site and 5' major splice donor site can readily be established by one skilled in the art by reference to the published SIV sequences or for example by aligning a variant SIV to the sequences set out and  
20 described herein.

In accordance with one aspect of the invention a SIV genome has a mutation within the packaging signal such that the SIV RNA is not packaged within the SIV envelope protein or capsid. Preferably such an SIV genome is capable of producing an SIV capsid.

25 Preferably, the packaging defective genome does not contain the SIV packaging sequences corresponding to the segments immediately downstream of the primer-binding site and just upstream of the 5' major splice donor and/or those immediately downstream of splice donor and immediately upstream of the *gag* gene. Typically, the vector may contain nucleotides ranging from about 20 bases of the  
30 primer-binding site to about 80 bases downstream of the primer-binding site and still be packaging-deficient and/or about 20 bases downstream of major splice donor to 70 bases downstream.

Preferably the packaging sequence absent from the vector comprises part of the region between the primer binding site and the 5' major splice site. In one embodiment, the packaging sequence absent from the vector in this region contains the 85-base nucleotide sequence shown herein as SEQ ID No. 2, i.e.

5 AGAACTCCTGAGTACGGCCTGAGTGAAGGCAGTAAGGGCGGCAGGAACC  
AACCACGACGGAGTGCTCCTATAAAGGCGCAGGTG.

The mutation may comprise a deletion of (a) the sequence of SEQ ID no 2, or (b) a fragment thereof of 5 or more nucleotides in length, or (c) a variant of either thereof. A variant of the sequence identified in SEQ ID No 2 is a corresponding 10 sequence derived from a variant SIV genome, which may be identified for example by identifying the major 5' splice donor site, primer binding site or gag initiation codon and aligning the sequence of the variant to SEQ ID No 2 to identify the corresponding sequence of the variant SIV genome to SEQ ID No 2.

In a preferred embodiment, the packaging signal comprises part or all of the 15 region of the genome 5' to the major splice donor site, for example a region commencing 90, 80, 70, 60 or 50 nucleotides upstream of (5' to) the 5' major splice donor site, extending to 40, 30, 20 or 10 nucleotides upstream of (5' to) the 5' major splice donor site. The mutation may comprise deletion or mutation within this region, for example to modify or delete 5, 10, 15, 20, 30, 40, 50 or 60 or more nucleotides 20 from this region. For example the packaging deletion may comprise nucleotides 53 to 85 of SEQ ID No 2.

This region of the SIV genome is the structural fold termed DIS and is 25 associated with a palindromic terminus. Preferably, the packaging sequences in this region are therefore mutated to disrupt the formation of the palindromic terminus and thus remove the DIS structure.

In another embodiment, other sequences, such as those downstream of the 5' major splice donor site extending up to the gag initiation codon are deleted. Preferably such sequences are deleted in addition to the mutation of sequences 30 upstream of the major splice donor. For example, the virus genome has an additional deletion or mutation in the 50-base segment sequence shown as SEQ ID No. 3, i.e. GAAATAGCTGTCTTACCAAGGAAGGGATAATAAGATAGATTGGGAGA T.

The number of bases that need to be deleted or mutated can vary greatly. For example, the given 50 or 85-base pair deletions in SIV are sufficient to result in loss of packaging ability. However, even smaller deletions in this region could also result in loss of packaging efficiency. Indeed, it is expected that a deletion as small as 5 about 5, 10, 15, 20 or 30 bases in this region and in particular a deletion in the region of nucleotides 53 to 85 of SEQ ID No 2, can remove efficient packaging ability. The size of a particular deletion can readily be determined based on the present disclosure by the person of ordinary skill in the art.

A mutation may comprise a deletion or modification of the sequence of SEQ 10 ID NO: 2. An appropriate modification may comprise a substitution, addition and/or deletion. An appropriate mutation will be one which leads to a reduction in the ability of viral RNA to be packaged within an SIV capsid. Preferably, such a mutation will lead to viral RNA not being packaged within an SIV capsid.

The mutation may alternatively comprise deletion or modification of a 15 fragment of SEQ ID NO: 2 or a variant thereof of 5 or more nucleotides in length. Such a fragment may be an internal fragment, that is to say, a deletion of 5 or more nucleotides within SEQ ID NO: 2, not including the end nucleotides of SEQ ID NO: 2. Such a fragment may be, for example, 5, 10, 15, 20 or 25 nucleotides in length. In the alternative, the fragment may comprise a fragment of 17 or more nucleotides in 20 length, selected from any portion of SEQ ID NO: 2 or a variant thereof including a terminal fragment thereof. Such a fragment may be, for example, 15, 25, 35, 45, 55, 65 or 75 nucleotides in length.

Alternatively larger deletions may be incorporated. Preferably, a larger 25 deletion will comprise the 85 base nucleotide region shown in SEQ ID NO 2 and will extend from this location in the SIV genome in one or both directions. Such a deletion may comprise a deletion of, for example, 1, 2, 5, 10, 20, 30, 50 or more bases at one or both ends of this sequence.

A variant of the sequence identified in SEQ ID NO; 2 is a corresponding 30 sequence derived from a variant SIV genome which may be identified, for example, by identifying the major 5' splice donor site, primer binding site or gag initiation codon of a variant SIV genome and aligning the sequence of the variant to SEQ ID

NO: 2 or to the sequence of the SIV genome to identify the corresponding sequence of the variant SIV genome to SEQ ID NO: 2.

The vector should contain an SIV nucleotide segment containing a sufficient number of nucleotides corresponding to nucleotides of the SIV genome to express functional SIV gene products, but as described above, should not contain a sufficient number of nucleotides corresponding to the region between the primer-binding site and the 5' major splice donor or between 5' major splice donor and *gag* gene to permit efficient packaging of the viral RNA into virions. In using these vectors to establish SIV packaging-defective cell lines, it is preferred that such cell lines do not produce any infectious SIV. Although a cell line transformed by these packaging-defective deficient vectors would have low infectivity because the cells are packaging-defective, some RNA can still be packaged into the virion. Accordingly, it is preferable that the SIV nucleotide segment does not correspond to the entire SIV genome so that, if some of the viral RNA is packaged into the virion, what is packaged will not be replication-competent virus.

The SIV genome as used herein refers to the viral RNA derived from an SIV. The simian immunodeficiency viruses (SIV) of the invention may be derived from any SIV strain, or derivatives thereof. Derivatives preferably have at least 70% sequence homology to the SIV genome, more preferably at least 80%, even more preferably at least 90 or 95%. Other derivatives which may be used to obtain the viruses of the present invention include strains that already have mutations in some SIV genes. Other mutations may also be present as set out in more detail below. The position of locations such as the primer binding site and 5' major splice donor site can readily be established by one skilled in the art by reference to the published SIV sequences or for example by aligning a variant SIV to the sequences set out and described herein.

#### Vectors comprising HIV-2 packaging sequences

The vectors comprising HIV-2 packaging sequences may be capable of being packaged by the SIV envelope or heterologous viral envelopes such as the Amphrotrophic Murine Leukaemia Virus envelope of the Vesicular Stomatitis Virus

G protein (VSV-G). These vectors may be capable of being packaged by HIV-1 and/or HIV-2.

The invention additionally relates to a vector for expression of a heterologous gene which may be packaged into the SIV genome through the use of 5 HIV-2 packaging sequences. Such a vector may comprise any suitable vector compatible with the proposed administration or use of the virus so long as HIV-2 packaging sequences are incorporated. Preferably the vector is derived from the HIV-2 genome but includes mutation in one or more HIV-2 genes, for example, to render the HIV-2 genome replication deficient.

10 A suitable HIV-2 vector should contain a sufficient number of HIV-2 nucleotides to permit efficient packaging of the viral RNA into virions.

HIV-2 has been described in a number of references. For example, McCann and Lever, J Virology 71: 4133-4137 (1997) disclose pSVR which is in an infectious proviral clone of the ROD strain of HIV-2 containing the replication origin of simian 15 virus 40. HIV-2 nucleotide positions herein are numbered relative to the first nucleotide of the viral RNA, that is, the transcript start site is defined as 1.

HIV-2 packaging sequences have also been described in the art (Griffin, S.D.C, J. Virol., 2001; 75: 12058-12069)

20 SEQ ID NO: 1 comprises positions 380-408 of the HIV-2 RNA and has been demonstrated as being important for packaging of HIV-2 in accordance with the present invention. The 28 based nucleotide sequence of SEQ ID NO: 1 is AACAAACCACGACGGAGTGCTCCTAGAA.

25 Preferably, a HIV-2 vector of the invention comprises an HIV-2 genome which comprises at least (a) a sequence of SEQ ID NO: 1 or a fragment thereof, (b) an internal fragment thereof of 5 or more nucleotides in length, or (c) a fragment thereof of 17 or more nucleotides in length.

30 A suitable vector may comprise a complete HIV-2 packaging signal or a sequence of SEQ ID NO: 1 comprising one or more deletions or modifications. An appropriate modification may comprise a substitution, addition and/or deletion. An appropriate mutation will be one which retains the ability of viral RNA to be packaged within an HIV-2 capsid.

The vector may alternatively comprise a partially deleted or modified fragment of SEQ ID NO: 1 or a variant thereof of 5 or more nucleotides in length.

Such a fragment is an internal fragment, that is to say, a deletion of 5 or more nucleotides within SEQ ID NO: 1, not including the end nucleotides of SEQ ID NO:

- 5     1. Such a fragment may be, for example, 5, 10, 15, 20 or 25 nucleotides in length. In the alternative, the fragment may comprise a fragment of 17 or more nucleotides in length, selected from any portion of SEQ ID NO: 1 or a variant thereof including a terminal fragment thereof. Such a fragment may be, for example, 17, 19, 21, 23, 25, or 27 nucleotides in length.

10       Alternatively larger portions of the HIV-2 genome may be incorporated.

Preferably, such a larger portion will comprise positions 380-408 of the HIV-2 RNA and will extend from this location in one or both directions. Such a portion may comprise, for example, 1, 2, 5, 10, 20, 30, 50 or more bases at one or both ends of this sequence. This region of the HIV-2 genome includes a proposed structural fold, 15 and is associated with a palindromic terminus. Preferably the deletion will allow the formation of the palindromic terminus. Preferably the vector will comprise a sequence lying between the primer binding site and this proposed structural fold.

A variant of the sequence identified in SEQ ID NO: 1 is a corresponding sequence derived from a variant HIV-2 genome which may be identified, for 20 example, by identifying the major 5' splice donor site, primer binding site or gag initiation codon of a variant HIV-2 genome and aligning the sequence of the variant to SEQ ID NO: 1 or to the sequence of the HIV-2 genome described in McCann and Lever (*supra*) to identify the corresponding sequence of the variant HIV-2 genome to SEQ ID NO: 1.

25       The HIV-2 genome as used herein refers to the viral RNA derived from an HIV-2. The human immunodeficiency viruses (HIV-2) of the invention may be derived from any HIV-2 strain, or derivatives thereof. Derivatives preferably have at least 70% sequence homology to the HIV-2 genome, more preferably at least 80%, even more preferably at least 90 or 95%. Other derivatives which may be used to 30 obtain the viruses of the present invention include strains that already have mutations in some HIV-2 genes. Other mutations may also be present as set out in more detail below. The position of locations such as the primer binding site and 5' major splice

donor site can readily be established by one skilled in the art by reference to the published HIV-2 sequences or for example by aligning a variant HIV-2 to the sequences set out and described herein.

Preferably the packaging sequences present in such a vector correspond to  
5 those described above which are mutated to produce a packaging defective HIV-2 vector. Preferably a substantial portion of the packaging signal is included. In a preferred aspect, the packaging sequence comprises the sequence of SEQ ID NO: 1, or a fragment thereof or a variant thereof. All of the HIV-2 sequences described above are preferred sequences for incorporation into a vector such that the vector can  
10 be packaged by an HIV-2 capsid or protein envelope.

In addition to the packaging sequences described above, further HIV-2 packaging sequences may be present in a vector. These sequences may comprise 10, 20, 50, 100, 200, 300 or 400 or more polynucleotides from a region downstream of the 5' splice donor site. In a preferred aspect, these packaging sequences comprise the  
15 5' part of *gag*, preferably comprising the matrix (MA) region of the *gag* ORF. In a preferred aspect, the packaging sequence comprises the sequence that lies between positions 553 and 912 of the HIV-2 RNA, or a variant thereof. A variant of such a packaging sequence is a corresponding sequence derived from a variant HIV-2 genome which may be identified, for example, by identifying the major 5' splice  
20 donor site, primer binding site or *gag* initiation codon of a variant HIV-2 genome and aligning the sequence of the variant to the sequence of the HIV-2 genome described in McCann and Lever (*supra*) to identify the corresponding sequence of the variant HIV-2 genome to SEQ ID NO: 1.

These vectors may be used as an extremely efficient way to package desired  
25 genetic sequences and deliver them to target cells. This may be done by preparing a vector containing a nucleotide segment containing a sufficient number of nucleotides corresponding to the packaging nucleotides of HIV-2 (HIV-2 packaging region), a predetermined gene and, flanking the packaging sequence and predetermined gene, sequences corresponding to a sufficient number of sequences from within and near  
30 the LTR for packaging, reverse transcription, integration of the vector into target cells and gene expression from the vector.

The packaging region preferably corresponds to at least the sequence of SEQ ID NO: 1. The vector might also comprise the 5' part of *gag*, preferably including the matrix (MA) sequence of HIV-2 in order to enhance packaging efficiency. For example, a sufficient number of HIV-2 sequences to be packaged, reverse-transcribed, integrated into and expressed in the target cells would include the U3,R and U5 sequences of the LTRs, the packaging sequences and some sequences flanking the LTRs (required for reverse transcription). Mutation of the *gag* initiation codon might be acceptable to avoid translation starting from this point whilst still retaining the *cis* acting *gag* nucleotide sequence required for packaging. For example, the *gag* ATG may be changed to ATC by site-directed mutagenesis.

When this vector is used to transfect an SIV packaging-deficient cell, it is the nucleotide sequence from this vector that will be packaged in the virions produced. These packaged genes may then be targeted to cells. This method of transformation is expected to be much more efficient than current methods. Further, by appropriate choice of genes, the method of infection may be monitored.

For example, the vector could contain a sufficient number of nucleotides corresponding to both 5' and 3' LTRs of HIV-2 to be expressed, reverse-transcribed and integrated, a sufficient number of nucleotides corresponding to the HIV-2 packaging sequences to be packaged. The vector would also contain a sufficient number of nucleotides of the gene which is desired to be transferred to produce a functional gene (e.g. gene segment). This gene can be any gene desired, as described below. The vector may also contain sequences corresponding to a promoter region which regulates the expression of the gene. The vector may be a self-inactivating vector, for example a self-inactivating retroviral vector. This may comprise a mutation in the U3 region of the 3'LTR of the vector which, after infection of the target cell during reverse transcription, is copied so that the 5' LTR contains this inactivating mutation, and the long terminal repeat promoter is inactivated. This leaves any internal promoter to function independently of any competition.

30 Host cells

In one aspect of the present invention, host cells are generated to produce SIV virus containing a vector for expression of a heterologous gene. The viruses are

produced by co-transfected a cell with a vector which is capable of producing an SIV capsid and a vector according to the invention having an HIV-2 packaging signal and a heterologous gene. In a preferred aspect, the vector which is capable of producing an SIV capsid is a packaging defective SIV vector according to the 5 invention. Such viruses are produced by co-transfected a suitable cell such as a mammalian cell with both vectors.

Preferably, a selected cell line is transformed using at least two different vectors. Then, by co-transfected a cell with each vector, the cell would still be able to express all the viral structural and enzymatic proteins and produce virions. The, or 10 each, vector may be a self-inactivating vector. This may, for example, comprise a mutation in the U3 region of the 3'LTR of the vector which, after infection of the target cell during reverse transcription, is copied so that the 5' LTR contains this inactivating mutation and the long terminal repeat promoter is inactivated. This leaves any internal promoter to function independently of any competition. Selection 15 of particular promoters and polyadenylation sequences can readily be determined based upon the particular host cell. Preferably the LTR to which the sequences do not correspond is the 3'LTR.

In one preferred embodiment, one vector includes sequences permitting expression of proteins upstream of *env* and the second vector permits expression of 20 the remaining proteins. For example, one vector contains a nucleotide segment corresponding to a sufficient number of nucleotides upstream of the *gag* initiation codon to the *env* gene sequence to express the 5'-most gene products. The other vector contains a nucleotide segment corresponding to a sufficient number of nucleotides downstream of the *gag* gene sequence and including a functional *env* 25 gene sequence. Such vectors can be chemically synthesised from the reported gene sequence of the HIV-2/SIV genome or derived from the many available HIV-2/SIV proviruses, by taking advantage of the known restriction endonuclease sites in these viruses by the skilled artisan based on the present disclosure.

Preferably, a different marker gene is added to each vector. Then, using a 30 preselected cell line cotransfected with these different vectors, and by looking for a cell containing both markers, a cell that has been cotransfected with both vectors is found. Such a cell would be able to produce all of the retroviral proteins. Although

virions would be produced, the RNA corresponding to the entire viral sequences would not be packaged in these virions. One can use more than two vectors, if desired, e.g. a *gag/pol* vector, a protease vector and an *env* vector.

Retroviruses can in some cases be pseudotyped with the envelope glycoproteins of other viruses. Consequently, one can prepare a vector containing a sufficient number of nucleotides to correspond to an *env* gene from a different retrovirus. Preferably, the 5'LTR of this vector would be of the same genome as the *env* gene. Such a vector could be used instead of an SIV *env* packaging-defective vector, to create virions. By such a change, the resultant vector systems could be used in a wider host range or could be restricted to a smaller host range. Using a vesicular stomatitis virus or rabies virus envelope protein would make the vector tropic for many different cell types.

Virtually any cell line can be used. Preferably, a mammalian cell line is used, for example CV-1, Hela, Raji, SW480 or CHO.

In order to increase production of the viral cellular products, one could use a promoter other than the 5' LTR, e.g. by replacing the 5' LTR with a promoter that will preferentially express genes in CV-1 or HeLa cells. The particular promoter used can easily be determined by the person of ordinary skill in the art depending on the cell line used, based on the present disclosure.

In order to enhance the level of viral cellular products, one can also add enhancer sequences to the vector to get enhancement of the LTR and/or promoter. Particular enhancer sequences can readily be determined by a person of ordinary skill in the art depending on the host cell line.

By using a series of vectors that together contain a complete retroviral genome (though a combination of HIV-2 and SIV sequences), one can create cell lines that produce a virion that is identical to the SIV virion except that the virion does not contain SIV RNA. These virions can readily be obtained from the cells. For example, the cells are cultured and the supernatant harvested. Depending on the desired use, the supernatant containing the virions can be used or these virions can be separated from the supernatant by standard techniques such as gradient centrifugation, filtering etc.

These attenuated virions are extremely useful in preparing a vaccine. The virions can be used to generate an antibody response to these virions. Pseudotyped virions produced from cell lines cotransfected with retroviral *gag/pol* and protease genes and containing the *env* gene from another virus may be useful in creating a 5 vaccine against this other virus.

#### Methods of mutation

Mutations may be made in HIV-2 or SIV by homologous recombination methods well known to those skilled in the art. For example, HIV-2 or SIV genomic 10 RNA is transfected together with a vector, preferably a plasmid vector, comprising the mutated sequence flanked by homologous HIV-2 or SIV sequences. The mutated sequence may comprise deletions, insertions or substitutions, all of which may be constructed by routine techniques. Insertions may include selectable marker genes, for example lacZ, for screening recombinant viruses by, for example,  $\beta$ -galactosidase 15 activity.

The number of bases that need to be deleted or mutated can vary greatly. For example, in SIV, the deletion of the 85-base pair sequence of SEQ ID NO 2 is sufficient to result in loss of packaging ability. However, even smaller deletions in this region could also result in loss of packaging efficiency. Indeed, it is expected 20 that a deletion as small as about 5, 10, 15, 20, 30, 40, 50, 60, 70 or 80 bases in this region can remove efficient packaging ability. The mutation may comprise deletion or modification of a fragment of SEQ ID NO: 2 or a variant thereof of 5 or more nucleotides in length. Such a fragment is preferably an internal fragment, that is to say, a deletion of 5 or more nucleotides within SEQ ID NO: 2, not including the end 25 nucleotides of SEQ ID NO: 2. Alternatively larger deletions may be incorporated as described above. The size of a particular deletion can readily be determined based on the present disclosure by the person of ordinary skill in the art.

Essential genes may be rendered functionally inactive by several techniques well known in the art. For example, they may be rendered functionally inactive by 30 deletions, substitutions or insertions, preferably by deletion. Deletions may remove portions of the genes or the entire gene. For example, deletion of only one nucleotide may be made, resulting in a frame shift. However, preferably larger

deletions are made, for example at least 25%, more preferably at least 50% of the total coding and non-coding sequence (or alternatively, in absolute terms, at least 10 nucleotides, more preferably at least 100 nucleotides, most preferably, at least 1000 nucleotides). It is particularly preferred to remove the entire gene and some of the flanking sequences. Inserted sequences may include the heterologous genes described below.

#### Heterologous genes and promoters

A vector or viruses of the invention may be modified to carry a heterologous gene, that is to say a gene other than one present in the HIV-2 or SIV genome. In particular the invention provides vectors which have HIV-2 derived sequences sufficient to allow packaging of the vector into a SIV capsid. The vectors may be derived from HIV-2 genomes, incorporating mutations or deletions in one or more HIV-2 genes, or may be derived from other expression vectors which are modified to incorporate HIV-2 packaging sequences. The term "heterologous gene" comprises any gene other than one present in the HIV-2 genome. The heterologous gene may be any allelic variant of a wild-type gene, or it may be a mutant gene. The term "gene" is intended to cover nucleic acid sequences which are capable of being at least transcribed. Thus, sequences encoding mRNA, tRNA and rRNA are included within this definition. The sequences may be in the sense or antisense orientation with respect to the promoter. Antisense constructs can be used to inhibit the expression of a gene in a cell according to well-known techniques. Sequences encoding mRNA will optionally include some or all of 5' and/or 3' transcribed but untranslated flanking sequences naturally, or otherwise, associated with the translated coding sequence. It may optionally further include the associated transcriptional control sequences normally associated with the transcribed sequences, for example transcriptional stop signals, polyadenylation sites and downstream enhancer elements.

The heterologous gene may be inserted into for example an HIV-2 vector by homologous recombination of HIV-2 strains with, for example, plasmid vectors carrying the heterologous gene flanked by HIV-2 sequences. The heterologous gene may be introduced into a suitable plasmid vector comprising HIV-2 sequences using

cloning techniques well-known in the art. The heterologous gene may be inserted into an HIV-2 vector at any location. It is preferred that the heterologous gene is inserted into an essential HIV-2 gene. Preferably the vector is derived from an HIV-2 genome, but includes deletion of one, two or several of the HIV-2 genes, up to the 5 minimal sequences of the HIV-2 genome to provide for packaging and expression of the heterologous gene.

The transcribed sequence of the heterologous gene is preferably operably linked to a control sequence permitting expression of the heterologous gene in mammalian cells. The term "operably linked" refers to a juxtaposition wherein the 10 components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequence.

The control sequence comprises a promoter allowing expression of the 15 heterologous gene and a signal for termination of transcription. The promoter is selected from promoters which are functional in mammalian, preferably human, cells. The promoter may be derived from promoter sequences of eukaryotic genes. For example, it may be a promoter derived from the genome of a cell in which expression of the heterologous gene is to occur. With respect to eukaryotic 20 promoters, they may be promoters that function in a ubiquitous manner (such as promoters of  $\beta$ -actin, tubulin) or, alternatively, a tissue-specific manner (such as promoters of the genes for pyruvate kinase). They may also be promoters that respond to specific stimuli, for example promoters that bind steroid hormone receptors. Viral promoters may also be used, for example the Moloney murine 25 leukaemia virus long terminal repeat (MMLV LTR) promoter or promoters of HIV-2 genes.

The HIV-2 LTR promoter, and promoters containing elements of the LTR 30 promoter region, are especially preferred. The expression cassette may further comprise a second promoter and a second heterologous gene operably linked in that order and in the opposite or same orientation to the first promoter and first heterologous gene wherein said second promoter and second heterologous gene are the same as or different to the first promoter and first heterologous gene. Thus a pair

of promoter/heterologous gene constructs may allow the expression of pairs of heterologous genes, which may be the same or different, driven by the same or different promoters. Furthermore, the product of the first heterologous gene may regulate the expression of the second heterologous gene (or vice-versa) under 5 suitable physiological conditions.

The expression cassette can be constructed using routine cloning techniques known to persons skilled in the art (see, for example, Sambrook *et al.*, 1989, Molecular Cloning - a laboratory manual; Cold Spring Harbor Press).

It may also be advantageous for the promoters to be inducible so that the 10 levels of expression of the heterologous gene can be regulated during the life-time of the cell. Inducible means that the levels of expression obtained using the promoter can be regulated. For example, in a preferred embodiment where more than one heterologous gene is inserted into the vector or HIV-2 genome, one promoter would comprise a promoter responsive to the expression of the second protein and driving 15 the heterologous gene the expression of which is to be regulated. The second promoter would comprise a strong promoter (e.g. the CMV IE promoter) driving the expression of the second protein.

In addition, any of these promoters may be modified by the addition of further regulatory sequences, for example enhancer sequences. Chimeric promoters 20 may also be used comprising sequence elements from two or more different promoters described above, for example an MMLV LTR/ HIV-2 fusion promoter.

The heterologous gene may encode, for example, proteins involved in the regulation of cell division, for example mitogenic growth factors, cytokines (such as  $\alpha$ -,  $\beta$ - or  $\gamma$ -interferon, interleukins including IL-1, IL-2, tumour necrosis factor, or 25 insulin-like growth factors I or II), protein kinases (such as MAP kinase), protein phosphatases and cellular receptors for any of the above. The heterologous gene may also encode enzymes involved in cellular metabolic pathways, for example enzymes involved in amino acid biosynthesis or degradation (such as tyrosine hydroxylase), or protein involved in the regulation of such pathways, for example protein kinases 30 and phosphatases. The heterologous gene may also encode transcription factors or proteins involved in their regulation, membrane proteins (such as rhodopsin),

structural proteins (such as dystrophin) or heat shock proteins such as hsp27, hsp65, hsp70 and hsp90.

Preferably, the heterologous gene encodes a polypeptide of therapeutic use, or whose function or lack of function may be important in a disease process. For example, tyrosine hydroxylase can be used in the treatment of Parkinson's disease, rhodopsin can be used in the treatment of eye disorders, dystrophin may be used to treat muscular dystrophy, and heat shock proteins can be used to treat disorders of the heart and brain associated with ischaemic stress. Polypeptides of therapeutic use may also include cytotoxic polypeptides such as ricin, or enzymes capable of converting a precursor prodrug into a cytotoxic compound for use in, for example, methods of virus-directed enzyme prodrug therapy or gene-directed enzyme prodrug therapy. In the latter case, it may be desirable to ensure that the enzyme has a suitable signal sequence for directing it to the cell surface, preferably a signal sequence that allows the enzyme to be exposed on the exterior of the cell surface whilst remaining anchored to cell membrane.

Heterologous genes may also encode antigenic polypeptides for use as vaccines. Preferably such antigenic polypeptides are derived from pathogenic organisms, for example bacteria or viruses, or from tumours.

Heterologous genes may also include marker genes (for example encoding β-galactosidase or green fluorescent protein) or genes whose products regulate the expression of other genes (for example, transcriptional regulatory factors).

Gene therapy and other therapeutic applications may well require the administration of multiple genes. The expression of multiple genes may be advantageous for the treatment of a variety of conditions.

25

#### Administration

The vectors, host cells and viruses of the present invention may thus be used to deliver therapeutic genes to a human or animal in need of treatment.

One method for administered gene therapy involves inserting the therapeutic gene into a vector of the invention, as described above. Subsequently, cells are co-transfected *in vitro* with a vector comprising the heterologous gene and the HIV-2 packaging sequences and a packaging defective SIV vector. Culturing the cells leads

to production of SIV viral capsids, into which the heterologous gene vectors are packaged through the HIV-2 packaging sequences. The resultant recombinant virus may be combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition. Suitable carriers and diluents include isotonic saline  
5 solutions, for example phosphate-buffered saline. Vaccine compositions, in which the heterologous gene encodes an antigenic peptide or protein may be formulated with adjuvants to enhance the immune response generated. The composition may be formulated for parenteral, intramuscular, intravenous, subcutaneous, intraocular or transdermal administration.

10 The pharmaceutical composition is administered in such a way that the virus containing the therapeutic gene for gene therapy, can be incorporated into cells at an appropriate area.

15 The amount of virus administered is in the range of from  $10^4$  to  $10^{10}$  pfu, preferably from  $10^5$  to  $10^8$  pfu, more preferably about  $10^6$  to  $10^7$  pfu. When injected, typically 1 to 10  $\mu$ l of virus in a pharmaceutically acceptable suitable carrier or diluent is administered.

20 The routes of administration and dosages described are intended only as a guide since a skilled practitioner will be able to determine readily the optimum route of administration and dosage for any particular patient and condition.

25

#### Assay Methodologies

The viruses of the invention can also be used in methods of scientific research. Thus, a further aspect of the present invention relates to methods of assaying gene function in mammalian cells, either *in vitro* or *in vivo*. The function of  
25 a heterologous gene could be determined by a method comprising:

- (a) producing virus particles comprising an SIV capsid and vector having a heterologous gene packaged via HIV-2 packaging signals, and
- (b) introducing the resulting virus into a mammalian cell line; and
- (c) determining the effect of expression of said heterologous gene in said  
30 mammalian cell-line.

For example, the cell-line may have a temperature-sensitive defect in cell division. When an HIV-2 strain comprising a heterologous gene according to the

invention is introduced into the defective cell-line and the cell-line grown at the restrictive temperature, a skilled person will easily be able to determine whether the heterologous gene can complement the defect in cell division. Similarly, other known techniques can be applied to determine if expression of the heterologous gene 5 can correct an observable mutant phenotype in the mammalian cell-line.

This procedure can also be used to carry out systematic mutagenesis of a heterologous gene to ascertain which regions of the protein encoded by the gene are involved in restoring the mutant phenotype.

This method can also be used in animals, for example mice, carrying so-  
10 called "gene knock-outs". A wild-type heterologous gene can be introduced into the animal using a mutant HIV-2 strain of the invention and the effect on the animal determined using various behavioural, histochemical or biochemical assays known in the art. Alternatively, a mutant heterologous gene can be introduced into either a wild-type or "gene knock-out" animal to determine if disease-associated pathology is  
15 induced. An antisense nucleotide could also be introduced using the virus particle of the invention to create in effect a knock-out animal.

Alternatively, the mutant HIV-2 virus of the invention may be used to obtain expression of a gene under investigation in a target cell with subsequent incubation with a test substance to monitor the effect of the test substance on the 20 target gene.

Thus, the methods of the present invention may be used in particular for the functional study of genes implicated in disease.

The invention will be described with reference to the following Example,  
25 which are intended to be illustrative only and not limiting.

### Example

We examined the cross packaging relationship between gene transfer and  
30 gag-pol constructs based on human immunodeficiency viruses type 1 and 2 (HIV-1 and HIV-2) and Simian immunodeficiency virus (SIV) using packaging vectors expressing Green Fluorescent Protein (GFP). Virion RNA was extracted using the

- Qiagen Rneasy system from 10ng of each lentiviral vector preparation, as measured by the reverse transcriptase assay (Cavidi Tech). The extracted RNA was first treated with DNase (Rnase free) for 15mins at 37°C and the DNase was then inactivated after incubation at 70°C for 10mins. The RNA was then reverse transcribed using the Promega ImProm-II reverse transcription system and an antisense GFP primer. The cDNA was serially diluted (1/10) in H<sub>2</sub>O and each aliquot was amplified by PCR using primers to GFP. The amplified products from four of the serial dilutions (1:10 to 1:10<sup>4</sup>) were electrophoresed on an a 2% agarose gel (Figure 1). Lanes 17 and 18 are negative controls and 19 is +ve GFP control
- The gene transfer efficiency of the cross-packaged viral vectors was assessed by transduction of 293T cells using a range of viral vector concentrations (20ng, 10ng, 5ng & 2ng). FACS analysis of GFP expression was performed 72 hours post transduction (Figure 2)..

HIV-1 gag-pol cross-packaged the genomic RNA of HIV-2 and SIV gene transfer vectors, however the packaging of SIV RNA was less efficient. SIV gag-pol was unable to package HIV-1 RNA but cross-packaged HIV-2 RNA at a similar level as the HIV-1/HIV-1 homologous vector system and also showed comparable levels of GFP gene by FACS analysis. HIV-2 gag-pol did not cross package either HIV-1 or SIV RNA. Efficient GFP gene transfer to rat glial and human embryonic CNS stem cells was shown for the cross-packaged HIV-1 /HIV-2 and SIV/HIV-2 viral vectors. Results are summarised in Table 1.

We demonstrate that HIV-1 gag-pol has a universal cross packaging ability among primate lentiviruses. This relationship is non-reciprocal with HIV-2 and SIV gag-pol unable to package HIV-1 RNA. Interestingly, the cross-packaged viral vector based on SIV gag-pol and HIV-2 RNA showed efficient gene transfer and may have potential for gene therapy in terms of bio-safety.

Table 1: Summary of results of Virion RNA PCR for GFP and FACS data on transduced cells with cross-packaged lentiviral vectors

Gag-Pol	Gene Transfer	RNA Packaged	GFP expression
---------	---------------	--------------	----------------

	<b>Vector (GFP)</b>	<b>(Limit of RT- PCR)</b>	<b>(Transduced cells)</b>
HIV-1	HIV-1	$10^3$	+++
HIV-1	HIV-1 (+cPPT)		++++
HIV-1	HIV-2	$10^3$	+++
HIV-1	SIV	$10^2$	+
SIV	SIV	$10^2$	+
SIV	HIV-1	$10^2$	-(neg)
SIV	HIV-2	$10^4$	++++
HIV-2	HIV-2	$10^3$	+++
HIV-2	HIV-1	$10^2$	-(neg)
HIV-2	SIV	$10^2$	-(neg)

## SEQUENCE LISTING

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CLAIMS

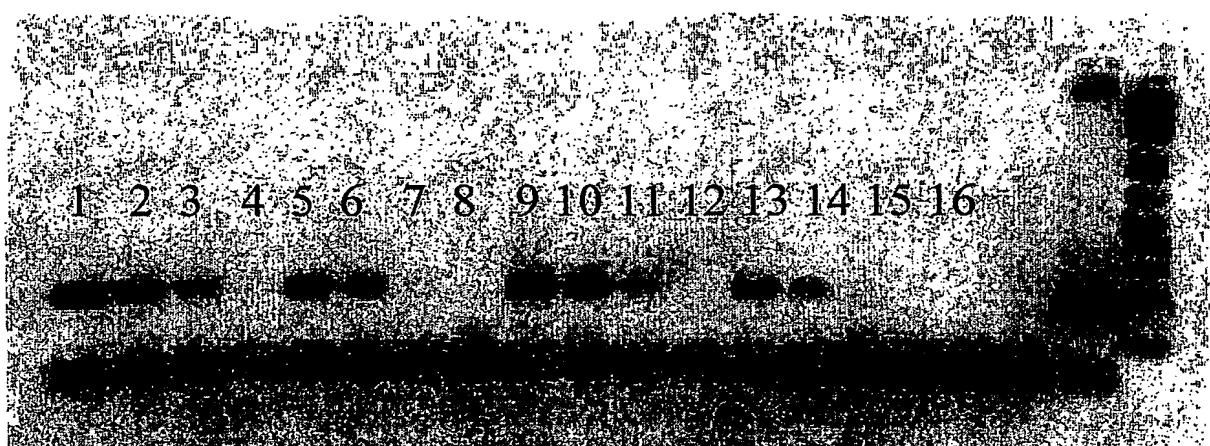
1. A process for producing a Simian Immunodeficiency Virus (SIV) encoding a heterologous gene, which process comprises infecting a host cell with a first vector which is capable of producing SIV capsid and a second vector comprising a Human Immunodeficiency Virus type 2 (HIV-2) packaging signal sufficient to package the vector in the SIV capsid and a heterologous gene capable of being expressed by the vector; and culturing the host cell.  
5
2. A process according to claim 1 wherein the first vector is a SIV vector comprising a mutation within an SIV packaging signal such that viral RNA is not packaged within an SIV capsid.  
10
3. A process according to claim 2 wherein said mutation comprises a deletion in the region between the primer binding site and the 5' major splice donor site of SIV.  
15
4. A process according to claim 2 or 3 wherein said mutation comprises a deletion within the DIS structure.
5. A process according to claim 2, 3 or 4 wherein said mutation comprises a deletion of:
  - (a) a sequence of SEQ ID NO: 2;
  - 20 (b) a fragment thereof of 5 or more nucleotides in length; or
  - (c) a variant of either thereof.
6. A process according to any one of the preceding claims wherein said second vector comprises:
  - (a) a sequence of SEQ ID no 1 or a variant thereof,
  - 25 (b) an internal fragment thereof of 5 or more nucleotides in length, or
  - (c) a fragment thereof of 17 or more nucleotides in length.
7. A process according to any one of the preceding claims wherein said second vector comprises the matrix (MA) region of the *gag* ORF or a fragment thereof.
- 30 8. A process according to any one of the preceding claims wherein said second vector comprises nucleic acids 553 to 912 of HIV-2 RNA or a fragment thereof.

9. A process according to any one of the preceding claims wherein the heterologous gene encodes a therapeutic protein or peptide, an antigen protein or peptide.
10. A virus produced by a process of any one of the preceding claims.
- 5 11. A pharmaceutical composition comprising a virus according to claim 7 and a pharmaceutically acceptable carrier.
12. A virus according to claim 10 for use in gene therapy.
13. A method of delivering a therapeutic or antigenic protein or peptide to an individual comprising administering to the individual an effective amount of a  
10 first and second vector as defined in any one of claims 1 to 9, a virus according to claim 10 or a pharmaceutical composition according to claim 11.

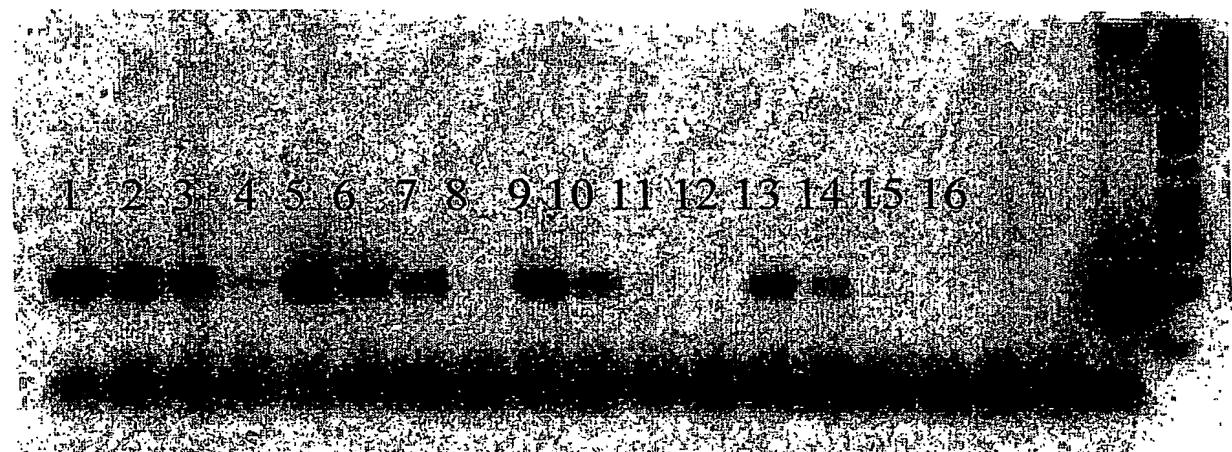
**ABSTRACT****CHIMAERIC VECTOR SYSTEMS**

5        A process for producing a Simian Immunodeficiency Virus (SIV) encoding a heterologous gene, which process comprises infecting a host cell with a first vector which is capable of producing SIV capsid and a second vector comprising a Human Immunodeficiency Virus type 2 (HIV-2) packaging signal sufficient to package the vector in the SIV capsid and a heterologous gene capable of being expressed by the  
10      vector; and culturing the host cell

**A**

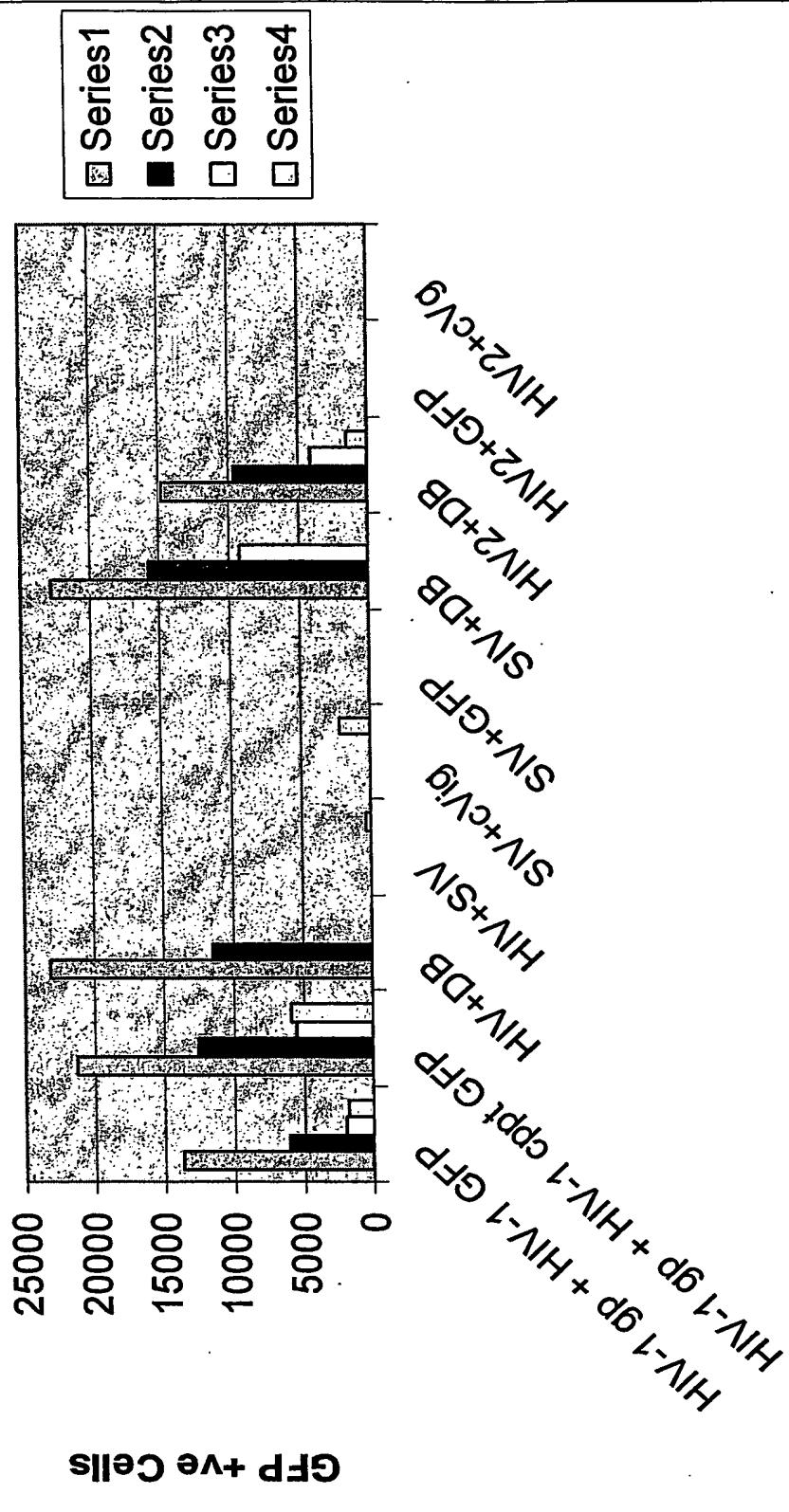


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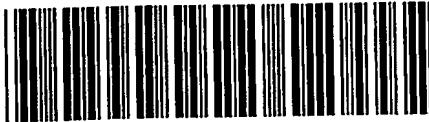
**Figure 1**

## Transduction of 293T cells



**Figure 2**

PCT/GB2004/003438



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